Search for the Cause

PROLOGUE In the previous learning experience, you were introduced to different causative agents of disease. What makes these organisms pathogenic? What special characteristics make these organisms harmful to their hosts? Is one factor responsible for the virulence of all these organisms, or many different factors? These are some of the questions scientists ask as they investigate infectious diseases. In this learning experience, you will analyze an important experiment carried out in an attempt to answer these questions, and conduct an experiment that investigates the factor(s) responsible for virulence in organisms.

Griffith's Search for the Cause

By the early 1900s, the notion that contagious diseases were the result of infectious agents had been accepted. From that time on, a major focus of research into infectious disease would be to understand the mechanisms by which these infectious agents caused the symptoms of disease in their hosts. In 1928, a British bacteriologist named Frederick Griffith was investigating the way in which a certain type of bacteria, *Diplococcus pneumoniae*, caused pneumonia, a serious and often fatal lung disease. Scientists already knew which type of bacteria caused the disease, but they were trying to learn how the bacteria caused the disease.

Griffith studied two strains of *D. pneumoniae.* Both grew very well in special culture media in his laboratory, but only one of them actually caused pneumonia when injected into mice. Griffith noticed that when he grew the bacteria on nutrient agar plates in the laboratory he could distinguish one strain from the other simply by its appearance on the agar. Each bacterium in the *virulent*, or disease-causing strain, secretes a polysaccharide (sugar)

coat called a *capsule* around its cell wall. Bacteria grow in colonies (a discrete mass of cells) on agar, and colonies of the virulent strain look smooth because of their capsules. Though Griffith did not know it then, we now understand that the capsules protect the bacteria from destruction by the host animal's immune response, allowing them to multiply and grow in the host. The other, *nonvirulent* strain Griffith studied did not produce capsules. Instead, when grown on agar the colonies, it appeared to have rough, jagged edges. The lack of polysaccharide capsules made this strain vulnerable to the immune system; when it entered a host it was destroyed by the immune response and was, therefore, nonpathogenic. (see Figure 4.1)

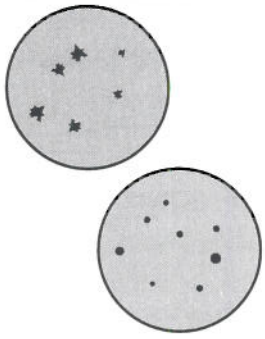
disease causing ability	appearance of agar plate	diagram of colony appearance on agar plate
nonencapsulated bacteria (does not cause disease)	rough edged colonies	
encapsulated bacteria (causes disease)	smooth colonies	

Figure 4.1
Summary of characteristics of the two strains of *D. pneumoniae*

Griffith hypothesized that the capsule might be responsible for the disease in some way. He had two important pieces of information (data) before he started the experiment: that the encapsulated bacteria could kill mice and that the nonencapsulated bacteria could not. With this information he was able to design a simple experiment providing a critical result, one that led investigators to the mechanisms by which these organisms can cause disease and acquire characteristics such as virulence. Figure 4.2 (on the next page) summarizes Griffith's experiments. Examine it and respond to the Analysis questions.

ANALYSIS

1. What simple question did Griffith pose in this experiment?
2. What parts of the experiment represented the controls? Describe how these served as controls.
3. If Griffith believed that the polysaccharide capsule of the encapsulated strain was responsible for its disease-causing characteristic,

what do you think he predicted would be the results of injecting the heat-killed, encapsulated bacteria alone? Why?

4. Why do you think the mouse died when Griffith mixed and injected the dead, virulent strain with the live, nonvirulent strain?
5. If you could isolate the bacteria that were injected as live, nonvirulent bacteria in the last experiment, how could you determine whether they had changed characteristics and had become virulent?

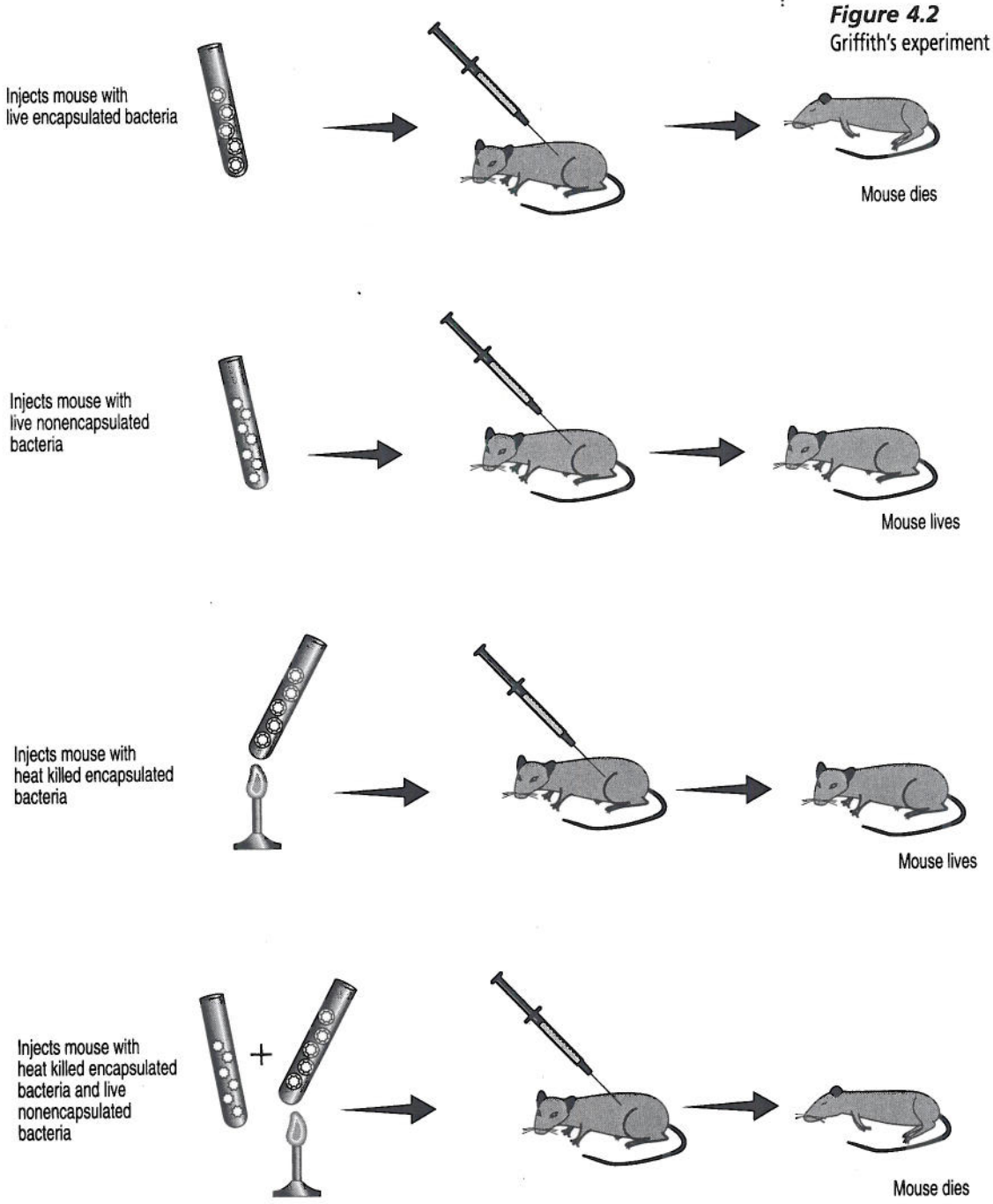


Figure 4.2
Griffith's experiment

ACTIVITY

In Isolation

INTRODUCTION When Griffith reisolated the originally nonvirulent strain from the dead mice in his experiment, he observed that this strain no longer formed rough colonies on nutrient agar plates, but formed smooth colonies. When these were injected into mice, the mice died of pneumonia. Somehow the nonvirulent, nonencapsulated strain had been changed, or transformed, into the pathogenic, encapsulated strain. Some principle or factor had been transferred from the killed, virulent strain to the live, nonvirulent strain, giving it the ability to make a polysaccharide capsule and cause disease.

For more than 15 years afterward, researchers attempted to identify what had happened in Griffith's experiment. What had changed the harmless strain into a virulent form? In 1944, Oswald T. Avery, Colin MacLeod, and Maclyn McCarty took the extract from dead virulent bacteria and one by one removed the biomolecules of the cell—first the proteins, then the carbohydrates, next the lipids, leaving the nucleic acids—each time testing the ability of that substance to make nonvirulent bacteria virulent.

In the following laboratory experiment you will use a procedure similar to that used by Avery, MacLeod, and McCarty to isolate the factor that transformed the harmless strain of bacteria into a virulent form. In your isolation you will be working with thymus or liver tissue rather than with *D. pneumoniae*. The same principles that Avery and his colleagues used to isolate the transforming factor apply to any living organism. Thymus and liver are more readily available, not virulent, and easier to work with than bacteria.

Before carrying out the actual isolation experiment, read the entire procedure in order to determine the principles behind the experiment.

MATERIALS NEEDED

For each pair of students:

- 2 pairs of safety goggles
- 1.0 mL of a homogenate of blended fresh thymus or liver (keep on ice)
- 2.0 mL salt (NaCl) solution
- 1 test tube (13 x 100 mm) with cap (or, cover with plastic wrap)
- 1 test tube rack (or small beaker to hold test tube)
- 5 mL ice-cold ethanol
- 1 glass stirring rod
- 1 ice water bath
- plastic wrap
- 2 10-mL conical centrifuge tubes with lids (optional)

For the class:

- 1 tabletop centrifuge (optional)

PROCEDURE

Use the Procedure Analysis column of Table 4.3, with the diagram of the cell that follows the table (Figure 4.4), in order to describe what is happening at the cellular level at each stage of the investigation. It is important to remember that these are the same principles Avery's team used when isolating the "transforming factor" from bacteria (they may have used different chemicals).

SAFETY NOTE: *Always wear safety goggles when conducting experiments.*

Table 4.3

PROCEDURE	PRINCIPLES INVOLVED	PROCEDURE ANALYSIS
1*. Blend together thymus with the buffer which contains: – sugar – aspirin – Epsom salts – water – detergent solution (*provided by teacher)	Detergent dissolves lipids and denatures proteins. Epsom salts and aspirin inactivate enzymes that degrade nucleic acid (DNA).	What does the detergent do to the cell? What parts of the cell are affected? Why are aspirin and Epsom salts added? What does the blending do?
2. Pour homogenate into a beaker. Place 1 mL in test tube or centrifuge tube. Add 2 mL of salt solution (NaCl and water) and shake well for two minutes.	Salt breaks up membranes further.	What effect is the salt having on the cell? Using the cell diagram, describe what has happened to the cell in steps 1 and 2.
3. If possible (this step is optional), spin tubes in a tabletop centrifuge for seven minutes. Be sure tubes are balanced. Remove tube from centrifuge and carefully pour off liquid into a clean test tube, being sure not to dislodge pellet.	High speed centrifugation separates larger structures such as membrane fragments from smaller, soluble biomolecules.	What is in the pellet? What is in the liquid?
4. Place test tube in an ice bath and leave for five minutes.	Cold temperatures slow down the action of enzymes.	Why is it advisable to keep the liquid cold?
5. Carefully pour or pipette 5 mL ice-cold ethanol down the side of the tube to form a layer on top of the water layer.	Nucleic acid is soluble in water but insoluble in ethanol.	What is happening when the ethanol is added?
6. Leave test tube undisturbed in ice water bath for 10 minutes.		

Continued on next page

PROCEDURE	PRINCIPLES INVOLVED	PROCEDURE ANALYSIS
7. After 10 minutes, dip the end of a glass stirring rod into the cell/ethanol mix. Slide the rod back and forth between the layers while spinning the rod with your fingertips.		
8. Place the material attached to the rod on a piece of plastic wrap. Roll it, stretch it, play with it.		What is the material on the glass rod?

Figure 4.4
Parts of an animal cell as seen under a microscope.

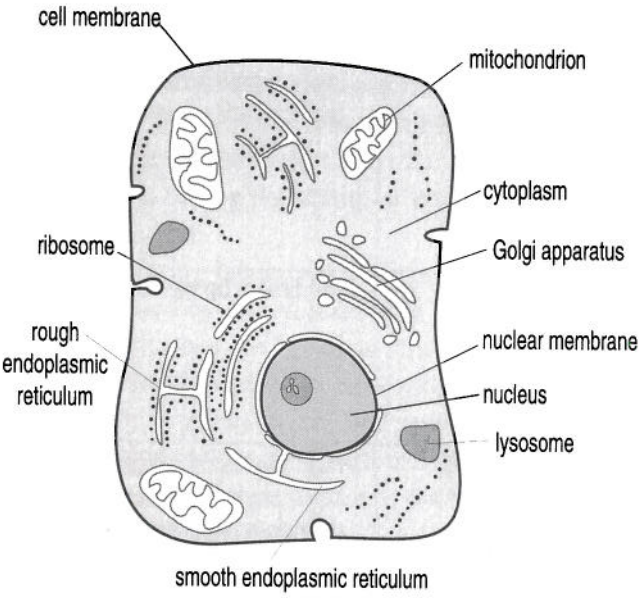


Table 4.5

CELL PART	BIOMOLECULE COMPOSITION
cell membrane	lipid, protein
endoplasmic reticulum	lipid, protein, nucleic acid
ribosome	protein, nucleic acid
Golgi apparatus	lipid, protein
lysosome	lipid, protein
cytoplasm	lipid, protein, nucleic acid, carbohydrate
mitochondrion	lipid, protein, nucleic acid, carbohydrate
nuclear membrane	lipid, protein
nucleus	lipid, protein, nucleic acid

ANALYSIS

1. In your notebook, diagram the components of a thymus or liver cell and indicate where the transforming material is located.
2. In a flow diagram, indicate what you think happens to the cell during the following stages of the procedure:
   - when adding the buffer solution and detergent;
   - when blending;
   - when adding the salt solution.
3. Describe why each biomolecule may or may not be a good candidate for the transforming property.
4. What is on your glass rod? Avery's team isolated this very same kind of material from the *D. pneumoniae* bacteria and determined that it was responsible for transforming the harmless bacteria. If what you have on your glass rod had been isolated from bacteria instead of thymus, how would you determine that this material was responsible for transforming the nonvirulent strain into the virulent strain? Design an experiment to show whether the harmless bacteria would be changed by this material.
5. Avery's team had isolated the material that could change an organism with one set of physical traits into an organism with a different set of physical traits. Explain how this material could be responsible for the "transforming property." Include what you know about this material and what you still need to know.

EXTENDING IDEAS

ON THE JOB

MEDICAL BACTERIOLOGY LABORATORY TECHNICIAN Would you be interested in using your organizational and detail-oriented skills working in the medical field? Medical bacteriology technicians conduct routine tests, which help to diagnose and treat disease, in bacteriology laboratories. A bacteriology laboratory technician can run a variety of laboratory procedures for identifying the presence of bacteria in body fluids and determining susceptibility to specific antibiotics. Technicians might prepare samples of body fluids or run laboratory tests such as urinalysis to diagnose bacterial contamination, or immune assays to detect the presence of viruses or bacteria. Technicians use light microscopes to view tissue samples and bacteria; sometimes they also use electron microscopes. This informa-

tion is used by physicians, surgeons or pathologists (people who study disease) to diagnose and treat patients. Laboratory technicians usually work in a hospital or in a laboratory affiliated with a medical practice. Medical laboratory technicians might have either one or two years of post-high school education in a medical lab's training program or a two year college degree in medical lab work. A four year college degree makes it possible to become a medical technologist, who can work on specialized or more complex work and supervise a laboratory. All laboratory technicians are certified. Classes such as biology, chemistry, math, computer science, and English are necessary.

SCIENCE WRITER With new infectious diseases and epidemics occurring around the world at an alarming rate, the job of a science writer has become closer to that of a war correspondent. Newspaper, magazine, television, and radio coverage of these events requires writers who are able to travel to other countries, report on events occurring there, translate technical scientific research and language into language that is more accessible to the general public. Good writing skills involve the readers and listeners in the drama of the moment while helping them to understand all the factors involved around crises such as epidemics. In addition to covering infectious diseases, science writers help the public keep abreast of new, exciting, and fascinating topics related to science. Besides writing for newspapers and magazines, a writer might choose to cover a topic in depth, writing about a specific discovery or spending a year in a research laboratory and writing a book about scientists, the scientific endeavor, and the topic being researched in that laboratory. Most science writers have a minimum of a college degree in a scientific discipline (although some have a liberal arts degree); they may also have a graduate level degree either in science, science journalism, or communications. Coursework in life sciences, chemistry, physics, English, and communications are recommended.